designed W-band loop-gap resonator, the sample is positioned in a gas-permeable Teflon tubing inside the resonator, which allows measurements of extremely small volume (~30 nL) of sample. We used this new design to measure properties of lens lipid membranes derived from total lipids extracted from both lenses (single donor) of a 2-year-old porcine cortex and nucleus. Detailed profiles of membrane fluidity and oxygen transport parameter were obtained from saturation recovery EPR. Analysis of conventional spectra using the microscopic-order macroscopic-disorder (MOMD) model provided rotational diffusion coefficients (R(perpendicular) and R(parallel)) and order parameters. Three different types of motion of lipid spin labels n-PC, T-PC, and CSL (ASL) with, respectively, nitroxide z-axis, x-axis, and y-axis parallel to the bilayer normal, are discussed. Results demonstrate that EPR at W-band has the potential to be a powerful tool for studying samples of small volume, ~30 nL, obtained from eye lenses of a single human donor.

3557-Pos Board B285

Phospholipid-Cholesterol Bilayers, Cholesterol Bilayer Domains, and Cholesterol Crystals were Detected in Lipid Dispersion Prepared from Lipids Extracted from Lens Nucleus of Old Human Donors

Laxman Mainali[1], Marija Raguz[1,2], William J. O'Brien[1], Witold K. Subczynski[1].
[1]Medical College of Wisconsin, Milwaukee, WI, USA, [2]University of Split, Split, Croatia.

Human lens lipid membranes prepared using a rapid solvent exchange method from the total lipids extracted from the clear lens cortex and nucleus of 61- to 70-year-old donors were investigated. The measured cholesterol-to-phospholipid (Chol/PL) molar ratio in these preparations was extremely high, showing values of 1.8 and 4.4 for cortex and nucleus, respectively. We expect that at this elevated Chol content, the entire membrane will become saturated with Chol (as in the case of the cortical membrane) and the excess of Chol will form Chol crystals, presumably outside the membrane (as in the case of the nuclear membrane). Properties and organization of the lipid bilayer were investigated using electron paramagnetic resonance spin-labeling methods. Formation of Chol crystals was confirmed using the differential scanning calorimetry. We showed that in the lipid dispersion prepared from nuclear lipids Chol exists in three distinguished environments: (1) Chol dispersed in PL bilayer, (2) Chol in non-crystalline membrane domains (cholesterol bilayer domains, CBDs), and (3) Chol in crystals. In cortical membranes, because of the lower Chol content, Chol crystals were not detected. Amounts of Chol in CBDs were almost the same in cortical and nuclear membranes which indicates that Chol content in both membranes is close or exceeds the Chol solubility thresholds in these membranes. Profiles of cortical and nuclear membrane properties (alkyl-chain order, fluidity, oxygen transport parameter, and hydrophobicity) were very similar to each other and to those reported for cortical and nuclear lens lipid membranes of 41- to 60-year-old donors reported earlier. This confirms our earlier statement that saturation with Chol determines properties of the PL bilayer with the minor effect of the PL composition.

3558-Pos Board B286

Be Careful When Choosing Your Dye Label: Commercial, Water-Soluble Fluorophores Often Interact with Lipid Bilayers

Robert J. Rawle, Laura D. Hughes, Steven G. Boxer.
Stanford University, Stanford, CA, USA.

Water-soluble small molecule fluorophores are widely used to label proteins, antibodies, lipids, etc. in biological systems. However, in a number of cases these fluorophores can interact strongly with lipid bilayers, influencing the interaction of the labeled target with the bilayer and/or producing misleading fluorescent signals. There is no quantitative, systematic measure of the extent of interaction between dye molecules and lipid bilayers. Here, we quantify the interaction of 32 commercially available water-soluble fluorophores with model lipid bilayers to aid in the selection of dye labels for fluorescence experiments. We also demonstrate that while calculations of a dye's hydrophobicity may be helpful in selecting a dye, those calculations are not robust in predicting the extent of dye-membrane interactions.

3559-Pos Board B287

Hydration and Temperature-Induced Phospholipid Phase Transitions and their Influence on Desiccation Tolerance of the Nematode Caenorhabditis Elegans

Sawsan E. Abusharkh[1], Cihan Erkut[2], Teymuras Kurzchalia[2], Karim Fahmy[1].
[1]Helmholtz-Zentrum Dresden-Rossendorf, Institute of Resource Ecology, Div. Biophysics, Dresden, Germany, [2]Max Planck Institute for Molecular Cell Biology and Genetics, Dresden, Germany.

The nematode Caenorhabditis elegans can bypass part of its normal larval development by forming a "dauer" state with arrested metabolism. The desiccation-tolerance of this state depends on the synthesis of trehalose during a preconditioning phase, where the worm is first exposed to mildly reduced RH, rendering the dauer state attractive for studying the physiology and genetics of Anhydrobiosis. Here, we correlate physical properties of phospholipids (PLs) with the desiccation tolerance of the larvae from which PLs were extracted. The response of PL structure to transient (seconds) changes in water potential and the role of trehalose in water-mediated structural transitions were addressed. Time-resolved Rapid scan FTIR spectroscopy was used in ATR geometry to record hydration-induced difference spectra. We show by chemical analysis that a reduction in choline content in the PL headgroup composition arises during preconditioning. This leads to a stronger coupling of headgroup hydration to disorder in PL acyl chains based on the time-resolved observation of PO2-, C=O and acyl CH2 stretching modes. Trehalose enhances this effect and leads to more uniform kinetics of hydration transients and lipid transitions. In combination with spectroscopic determination of altered lipid main phase transition temperatures (Tm) in PLs upon preconditioning, the data show that chemical tuning of the kinetics and the extent of coupling of headgroup hydration to acyl chain packing changes is a key process in desiccation tolerance as it may release mechanical stress from membranes during temporal changes of water potential. Additional DSC, ITC and Langmuir-Blodget data show that trehalose interacts more favourably with PLs from preconditioned larvae to support desiccation tolerance. This work highlights that chemical tuning of lyotropic phase transitions plays a fundamental role in the trehalose-dependent desiccation tolerance of C. elegans larvae.

3560-Pos Board B288

Physical Aspects of the Cut-Off Effect of N-Alcohols in Pure Lipid Membranes

Francisco J. Sierra-Valdez, J.C. Ruiz-Suárez.
Biophysics of Membranes, CINVESTAV, Apodaca, N.L., Mexico.

Nowadays, the molecular nature of general anesthetic target sites remains unknown; some theories profess that the action occurs in proteins, others in lipids. In particular, for nearly a century it has been known that alcohols can act as general anesthetics. However, as the chain length of an alcohol increases, so does its potency as an anesthetic, only up to a certain chain length beyond which the anesthetic activity disappears (the so-called "cut-off effect"). In the attempt to explain such phenomenon, and based on the proposed anesthetic theories, different explanations have emerged without conclusive arguments, nevertheless, the lack of sufficient evidences supporting the cut-off effect of n-alcohols in general anesthesia make this work worth to be pursue. In the present work, using calorimetry and atomic force microscopy (AFM), we show a systematic study of the interaction of n-alcohols (from methanol, C1, to eicosanol, C20) with lipid membranes, in order to collaborate in the comprehension of a physical mechanism of the cut-off phenomenon. Our results suggest that the lowering of the melting transition temperature (Tm) of lipid membranes due to short-chain alcohols, is highly related to their ability to disturb lipid membranes (as has been shown for a wide variety of anesthetics), whilst, the increase of Tm induced by long-chain ones (from C12), is caused by a stiffening of the lipid membrane. We also correlate such effects with some physical properties of n-alcohols and the lipid composition. These results concur with other findings to underwrite the idea that anesthesia does not need a specific binding site in a protein and allow us to speculate that anesthesia only depends on the ability of certain atom or molecule to solubilized in lipids increasing the disorder of the membrane.

3561-Pos Board B289

Interaction of Novobiocin with Salmonella Sp Outer Membrane

Thatyane M. Nobre[1], Michael Martynowycz[2], Tonya Kuhl[3], David Gidalevitz[2], Hiroshi Nikaido[1].
[1]University of California, Berkeley, Berkeley, CA, USA, [2]Illinois Institute of Technology, Chicago, IL, USA, [3]University of California, Davis, Davis, CA, USA.

Gram-negative bacteria possess a double membrane system, with the external leaflet of the outer membrane composed of lipopolysaccharides (LPS) that act as the first barrier to drugs. The importance of studying the permeability of LPS barrier comes from its correlation with strain susceptibility. In this study, we extracted LPS from two isogenic Salmonella sp strains, that contains alterations in the lipid A portion regulated by the phoPQ-system. Interaction between phoP- (LPS with no modification) or phoPc (palmitoylation of the 3-hydroxyl group in the 3-OH-myristyl residue, addition of 4-aminoarabinose to the 4' phosphate group and addition of a 2-hydroxy group to the myristate residue at the position 3') LPS and novobiocin was evaluated by using Langmuir monolayers. Novobiocin was chosen due to its size (612 Da), large enough to permeate cells throughout the LPS, and not through the porin channels. MIC of novobiocin for phoP- was 14 μg mL-1 and for phoPc, 40 μg mL-1. p-A isotherms indicated that phoP- occupies a limiting area of 150Å2,